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ON THE PRESENT STATE OF POLISH RESEARCH ON BRUCELLOSIS

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Reservoirs of Brucellosis in Poland

In our country, cattle are the brucellosis reservoir and, in the light of our observations and research, are practically the sole source of human infection. Despite extensive research in various sections of the country, we have not, up to now, been able to demonstrate the existence of brucellosis among sheep, goats, or swine. It may be that in our country, these animals do not play a very great role as reservoirs of brucellosis. This matter, however, requires further verification. Only sporadic cases of brucellosis among horses have been reported by Anczukowski and Szaflarski. Neither our investigations of many herds in which mares have aborted, nor mass investigations of horses on PGRs (Państwowe Gospodarstwa Rolne, State Farms) where there were cattle suffering from brucellosis, have revealed the infection among horses. Brucellosis among fowl, dogs, rodents, rabbits, and other animals has rarely been confirmed. Investigations of rodents killed on PGRs where cattle were infected with brucellosis have yielded very few positive serological results. Further research on rodents (rats, house mice, field mice, and forest mice) is required. Initial research on arthropods parasitizing cattle infected with brucellosis have not yielded positive results. This research will be continued.

The Brucellosis Microorganism

The former statistical division of Brucellae into separate, individual species, i. e., *Br. abortus bovis*, *Br. melitensis*, and *Br. suis*, no longer prevails. At present, we distinguish the following variants of the species *Brucellae*: *bovis*, *suis*, and *melitensis*. Extensive research has confirmed the correctness of the thesis that these variants are not stable and that in response to environmental changes, they are able to transfer from one host to another and can undergo transformations. A correlation can be established between the ecological characteristics of a geographic area and the appearance of a certain variant. *Br. melitensis* appears mainly in the Mediterranean basin, in the southeastern areas of the USSR, in southern France, etc. *Br. bovis* is distributed throughout Europe, Asia, and North America in areas corresponding to our latitude. *Br. suis* appears in the US, Denmark, and the USSR. This principle, however, can not be considered statistically invariable. Sheep transported from the south to the north of the USSR carry *Br. melitensis* with them, and these organisms are able both to adapt to the changed ecological conditions and to preserve their vitality, virulence, and infectiveness for humans and animals. Thus, the theory concerning the so-called mild virulence and infectiveness of *Br. bovis* can no longer be maintained. An increase in the virulence of *Br. bovis* has been observed. *Brucellae* isolated from humans as a variant of *Br. bovis* can not be distinguished from the virulent *melitensis* variant.

Which variant do we encounter in Poland?

Only the cattle variant was reported before the war. At that time, our collection of *Brucella* strains comprised about 160 native strains and the standard variants: *bovis*, *melitensis*, and *suis*. We shall attempt to describe the variants found in our country. Up to now, our investigations have disclosed only cattle variants.

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The mutability of Brucellae is very great. They appear, in S, R, SR, M, I, and other forms. The R form is the most important one for the selection of vaccine strains, for serological purification, for the production of allergen, and for the production of vaccines. The others are not suitable for diagnostic purposes or for the production of vaccines. As a basis for many experiments, we found Brown's method of thermoagglutination and Daszkiewicz's method of acriflavine agglutination to be the most advantageous for laboratory work.

The Immunology of Brucellosis

The works of Zdrodovskiy, Burnet, Huddleson, and others serve as the foundation of our knowledge concerning the immunology of brucellosis. The works of Zdrodovskiy and his followers, which are based on a materialistic methodology and the theories of Pavlov, are especially valuable. From these works, we have derived our knowledge of the dynamics of the development of the infection and of the immunological processes which occur during the course of brucellosis.

In numerous experiments carried out by Stepkowski, we have demonstrated the correctness of the propositions advanced by Zdrodovskiy's school.

Three general stages can be discerned in the course of the infection and immunological processes during brucellosis: the first, the acute period, when the infection is developing, (septicemia, fever, infectious toxicosis); the second, a subacute period, in which these conditions are less severe; and the third, a protracted period characterized by a chronic, symptomless, mildly developing infection, which is accompanied by organic changes.

During the first period, antibodies, i.e., agglutinins, precipitins, antitoxins, and complement fixation substances form the basis for defense. In the second period, the humoral defensive phenomena and the serological reactions are exhausted. Tissue, histocytic, and allergic defenses then manifest themselves. Phagocytic phenomena and allergodermal reactions form the basis of the organism's struggle against the infection during this period and assist in confirming the diagnosis of brucellosis. In the third period, brucellosis resembles the group of protracted diseases characterized by allergic conditions and premunition, i.e., tuberculosis, glanders, tularemia, malaria, etc. We have proved that the antibodies peculiar to brucellosis are one and the same substance (the unity theory), and that the pronounced phagocytosis in the second and third stages is not exclusively dependent on the operation of opsonins but rather on the phagocytes released by the action of the antibodies, which have independent phagocytic action (Parnas, Sluczanski). We have demonstrated that in agreement with Pavlovian science, the nervous system has a basic influence on the course of development of the immunological processes. Research on the influence of hypoglycemic shock (Wegierko), adrenalin, vitamin C, etc., has shown the effect of such agents through the nervous system on serological, allergic, and phagocytic reactions (Parnas, Zebracki, Kurancowa). The effect of antihistamine compounds on Burnet's allergodermal reaction has also been examined (Parnas, Daszkiewicz).

Knowledge of the immunological phenomena encountered in the course of brucellosis allows us to establish a suitable basis for the diagnosis and prevention of brucellosis by means of vaccines.

Burnet's phenomenon corresponds to Koch's phenomena in tuberculosis. By the intraperitoneal injection of a suspension of Brucellae into uninfected guinea pigs, we produced septicemia and a generalized infection with localization of very significant changes (and of the bacteria themselves) in the spleen, liver, lymphatic system, and bone marrow. This demonstrates the guinea pigs' lack of resistance. If we inject a suspension of Brucellae intraperitoneally into guinea pigs already infected with the bacteria, we observe the appearance of a defensive reaction in the peritoneum and in the neighboring portions of the lymphatic system (peritonitis sero-fibroso-purulenta). The masses of phagocytes accumulated in the peritoneal cavity display a high degree of phagocytic activity (60 or greater, according to our observations). This is an indication of their interinfectious immunity.

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We obtained similar results by injecting brucellin PS or PD intraperitoneally. Burnet's phenomenon has become the basis for diagnosing brucellosis through allergy reactions caused by injecting various brucellin allergens intracutaneously, subcutaneously, intraconjunctivally, supracutaneously, and intracutaneously into the eyelid.

Living vaccines have been developed which confer immunity on humans and animals (Zdrodovskiy's innocuous vaccine, No 19 Buck-Cotton vaccine, and Parnas' PD vaccine).

The Epidemiology of Brucellosis in Poland

The first human cases of brucellosis in Poland were described before the war by Legezynski, Karwacki, and Meisel. There were sporadic cases. One can believe that our prewar statistics represented only a fraction of the actual number of cases of brucellosis in Poland. The following factors may account for this: (1) the diagnosis of brucellosis was based on an agglutination reaction, and there were no country-wide standards for this reaction; (2) lack of sufficient knowledge of brucellosis among practicing physicians, epidemiologists, and clinicians; (3) lack of research and applied work on detecting brucellosis in the country; (4) underestimation of the significance of the state of health of agricultural workers in the villages; (5) underestimation of the problems involved in occupational diseases in rural areas and in the manufacturing industries; and (6) underestimation of zoonotic diseases in general.

In the new Poland, the situation is exactly the opposite.

All the above-mentioned problems have been resolved by the people's health service. The following observations on work on brucellosis conducted between 1944 and 1954 are presented here.

It appears that brucellosis occurs much more frequently, especially in rural areas, than had previously been estimated. The Clinical Section for Rural Occupational Diseases of the institute (Prof Dr A. Tuszkiewicz) has 80 brucellosis patients undergoing treatment at the clinic at present. But how many subclinical or symptomless cases of brucellosis are there throughout the country? We have often tried to answer this question.

The detection of a larger number of brucellosis cases than were reported before the war is due to the following reasons: (1) the improvement of the methods of diagnosing brucellosis and increased interest in this disease on the part of sanitary-epidemiological stations, clinics, and institutes (Marine Medicine and the State Institute of Hygiene); (2) a rise in the level of knowledge concerning brucellosis among practicing physicians, clinicians, and scientific workers; (3) the establishment of research centers for human and animal brucellosis, with close medical and veterinary cooperation; (4) the transfer of research from the laboratories to rural areas, PGRs, and Producer Cooperatives (the work of the Division of Anthropozoonosis of the Institute of Rural Medicine); and (5) a possible increase in the virulence of the infective organism.

The reason for this is that there are still a certain number of cases of brucellosis which remain unreported, or are wrongly diagnosed, due mainly to a lack of interest in the matter.

In view of the data accumulated by our institute, the occupational nature of brucellosis should be recognized. Veterinary physicians, veterinary feldshers, sanitary technicians, zootechnicians, milkmaids, stable hands, workers in the meat industry, laboratory workers, and individual peasants suffer from brucellosis. Veterinary and zootechnical personnel become infected through cattle during the investigation of cows for sterility (per vaginam), while assisting at births, and in

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treating leukorrhea and other gynecological diseases. Primarily young veterinary physicians, zootechnicians, and assistant personnel become infected. This is an indication of their disregard of precautionary measures, nonobservance of hygienic principles, careless work, and their high susceptibility to brucellosis. There have been cases of brucellosis among physicians and feldshers who were investigating either meat or workers in slaughterhouses. The possibility of infection per os must not be disregarded either. This, according to Gromashevskiy, can occur both during or after work by transporting the bacteria to the lips on contaminated hands while smoking cigarettes or eating. Investigations have shown the possibility of infection from the unprocessed hides of animals that had come in contact with the bacteria during breeding (Parna, Glinkowa). In one case, a veterinary physician became infected by accidentally puncturing his hand with a needle while vaccinating calves against brucellosis. It is a known fact that two students in the US developed a typical brucellosis infection after drops of the nonvirulent S 19 vaccine were introduced into their conjunctival sacs. The brucellae may have been rubbed into the conjunctiva as a result of rubbing the eyes while engaged in veterinary work. Milkmaids become infected through the skin or perorally. This pertains generally to agricultural workers and workers in the meat industry, to whom the bacteria gain entrance not only through the hands, but through the soles of the feet, i.e. by walking barefoot through manure in the slaughterhouses, dairies, etc. We have not observed any cases in which the infection was passed from one person to another. The children examined by us and at the clinic of Prof Baranski were presumably infected through milk.

Research on the appearance of brucellosis on FGRs among members of the veterinary service and among workers in the meat industry has yielded amazing results. Serological investigations of veterinary workers in Slask (Kaminska-Szaflarski), in Gdansk Wojewodstwo (Blawat), and in Wroclaw Wojewodstwo (Sobiech); survey investigations of veterinary physicians (Makowiec); serological and allergic investigations of veterinary physicians, veterinary feldshers, and sanitary workers of many powiats (Parnas) have disclosed a significant number of persons who gave positive serological and allergy reactions (up to 30%) but who did not exhibit any of the visible clinical symptoms of brucellosis. Clinical, serological, and allergy investigations of workers, which were carried out on FGRs in Lublin and Olsztyn wojewodstwow and which indirectly showed the state of the infection among cattle, yielded positive results, thereby disclosing persons infected with brucellosis (primarily, subclinically or symptomlessly). Teams of physicians and veterinary physicians carried out investigations on farms maintaining cattle infected by brucellosis. Investigations carried out by the institute at meat plants in Lublin, Stalinograd, and Warsaw (Parnas, Freytag) revealed a large number of workers who reacted positively to brucellin P3. Clinical investigations of these workers revealed no actual symptoms of brucellosis.

An interpretation of the results of this research gives rise to the following possibilities:

1. The positive Burnet reactions indicate that workers coming in contact with materials containing substances antigenic to Brucellae are sensitive to brucellin allergen. Experiments conducted by us on rabbits revealed no cases whatsoever of allergenic sensitivity, despite contact (cutaneously or intravenously) with killed Brucella substances. Likewise, we have not been able to detect the presence of anti-Brucellosis antibodies in the blood of the rabbits, despite their being fed on milk containing up to 100 ml [probably mlrd, meaning milliard (billion)] killed Brucellae per milliliter. Thus the proposition that the appearance of a positive agglutination reaction is due to prolonged use of boiled milk containing killed Brucellae must be rejected. It must, likewise, be accepted that the positive serological and allergodermal reactions, which are observed among workers who have been in contact, occupationally, with materials infected by Brucellae, are the result of infection by living bacteria.

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2. Positive Burnet reactions accompanied by negative serological reactions may signify a cure and the annihilation of the bacteria. The sequel of that process may be a state of sensitivity and the presence of antibodies. To verify this, we made experimental investigations on rabbits and guinea pigs which had been cured of a brucellosis infection by the use of streptomycin, sulfadiazine, chloromycetin, and aureomycin. As a result of the cure and the annihilation of the bacteria, serological reactions ceased entirely, and allergic reactions became weaker and weaker and finally disappeared. This indicates that the disappearance of the living organisms from the system leads subsequently to the cessation of serological reactions and a decrease in the characteristic sensitivity to brucellin. It must be concluded from this that a positive Burnet reaction is always an indication of an infection, which, depending on the massiveness of the infection, the virulence of the Brucellae, and the immunological condition of the organism, will run its course clinically, subclinically, symptomlessly, or latently, and will be accompanied by slowly developing reactive and organic metabrucellosis changes (Parnas, Stepkowski).

The Diagnosis of Brucellosis

The diagnosis of brucellosis in humans depends on the following groups of investigations:

1. Epidemiological investigations which take into consideration the place of work and contacts with animals suffering from brucellosis, or the animals' products, i.e., milk, meat, manure, hides, etc.
2. Clinical and supplemental investigations.
3. Laboratory investigations such as blood, agglutination reactions, complement fixation reactions, precipitation reactions, Coombs reactions, the determination of the phagocytic index, Burnet allergic reactions, and blood and marrow cultures.
4. In the case of men whose testicles are affected, bacteriological and serological investigations of the sperm must be carried out. In the case of women, after a miscarriage, investigations of the embryo, placenta, vaginal exudates using Holtha's antigen reaction and biological investigations must be carried out. In the case of women who are nursing, the milk is examined and ring reactions, serological reactions, and biological investigations are carried out.

Individual reactions were investigated by us and standardized.

Some physicians pay little attention to the place of work, while other physicians, veterinary physicians, zootechnicians, etc., often ask whether the disease is connected with the place of work. Clinical and hematological investigations, supplemented by radiological examinations of the chest cavity and spinal column and functional examination of the organs (liver), were carefully worked out by Tuszkiewicz and Szwedowski. An agglutination reaction is of decisive diagnostic significance if executed properly. We have worked out instructions for standardizing this reaction. Agglutination titers of 1/100 or 1/200 can be considered conclusive, the only exception being that during the course of typhus or tularemia, para-agglutination with Brucellae may occur. Then, Castellani's agglutinin reaction and a complement fixation reaction using rickettsial or tularemia antigens clarifies the matter. A titer of 1/50 or 1/25 is extremely doubtful. It can be confirmed by complement fixation reactions as brucellosis. A positive complement fixation reaction is grounds for considering both reactions as positive. Legczynski and Grycz had already carried out complement fixation reactions to confirm agglutination reactions before the war here in Poland. This method was not put into practice in the laboratories. Just after the war, this reactions became

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an indispensable part of serological research. There are cases where both reactions turn out negatively, despite the presence of clinical symptoms which cause the investigator to suspect brucellosis. This enters the region of complete retention of antibodies, agglutinins, and complement (or partial blockade). We, therefore, used a precipitation reaction with a suitable polysaccharide allergen (Parnas, Tuszkiewicz).

As is evident, in very complicated cases of brucellosis, it is necessary to carry out a serological analysis and on the basis of this to determine the serological agent in every case of brucellosis. We made cultures from blood and marrow in every case of brucellosis. Despite careful culturing and further biological investigation, we only succeeded in confirming the presence of Brucellae in two cases. The culturing must be repeated and must be carried out during the febrile period. A determination of Huddleson's phagocytic index is always made in human brucellosis cases. This test must be carried out in the clinic. High phagocytic activity (greater than an average of 20-40 per cell) leads to immunity and is a measure of the defensive strength of the system, taking into consideration the fact that a high level of antibodies and a positive Burnet reaction can not lead to a state of immunity.

Burnet's allergodermal reaction, to which we devoted extensive research, is of much greater diagnostic significance. A brucellin allergen must possess the following qualities: (a) sensitivity, reaction specificity; (b) lack of toxic activity; (c) lack of the ability to produce specific antibrucellosis antibodies; (d) permanence; (e) ease of production; (f) the possibility of use for vaccine therapy purposes.

The allergen used in Poland before the war (Zylbertal, Teklinski, Grycz) consisted of suspended, killed Brucella cells (Br. abortus) which were not suitable, since they did not possess the above-mentioned qualities. Brucellin PS (Parnas, Stepkowski) was developed in 1945, using a method of multiple freezing and thawing and mechanical destruction of the cells. Large concentrations of the bacteria, i.e., about 4 billion per milliliter, caused the appearance of toxic symptoms which were sometimes violent and unpleasant for both the patient and the physician. The dilution of Brucellin to one billion per milliliter produces a preparation completely suited for practical use. Investigations carried out at various clinics on about 500 patients free of brucellosis demonstrated the great specificity of this allergen, which, when used simultaneously on persons suffering from brucellosis, caused a very sensitive reaction. Brucellin PS is suitable for use in vaccine therapy, since it is a complete antigen which causes the production of antibodies in patients free from brucellosis and raises the reaction titers in infected persons. The disadvantage of using brucellin PS is that serological investigations can not be carried out on people who have used it for at least 8 weeks. Thus, in investigating a person suspected of having brucellosis, blood must be taken for serological reactions before the Burnet reaction is attempted.

The following work has been done on finding still better allergens:

1. The destruction of a suspension of Brucellae with an ultrasound apparatus (Brucellin, PD)
2. The obtaining of Brucella fractions, i.e., protein, polysaccharide, and protein-polysaccharide fractions
3. Comparative investigations of the Danish brucellin, "Peba" (Ottosen and Plum), which is similar to the Soviet preparation, "brucellohydrolyzate."

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It turned out that our fractions, obtained by chemical means, were less sensitive as allergens but caused the production of antibodies specific for Brucella. Feba turned out to be a nonantigenic, haptenic substance, the presence of which did not cause the production of antibodies. Feba is 100% less allergenic than our brucellin PS or PD and, for our purposes, is not suitable for application to either humans or animals. Brucellin PD turned out to be the best allergen that can be recommended at present. The following unified instructions have been worked out for the use and evaluation of brucellin PS and PD:

1. A total of 0.05 ml (3-4 drops) of brucellin PS should be injected intracutaneously into the inner side of the left forearm. The skin should be cleansed before the injection with either ether or alcohol but not with iodine. One drop of brucellin PS is sufficient for children. Airtight, sterile syringes and very small, short needles should be used. Subcutaneous injection of brucellin PS is pointless. Intracutaneous injection is always indicated by the appearance of a small bump in the skin at the injection site. If the intracutaneous injection does not take, it can be repeated on the other arm.
2. Brucellin PS must be kept in a dark, cool place. The ampules should be opened sterile by heating the glass and filing with a sterile file.
3. The injection site should be observed after 24 hours, 3, 4, and 5 days, and the perceptible general and local symptoms should be recorded.

Evaluation of the reaction:

- 0 Indicates no change in the skin except an insignificant, impermanent hyperemia.
- +
- ++ Indicates severe redness and swelling of an area 2 cm by 2 cm or more (a positive result).
- +++ Indicates severe redness and severe swelling of an area greater than 2 cm by 2 cm (a strong positive result).
- ++++ Indicates very severe redness, very severe swelling, a swollen center, and necrosis. The reaction extends on the surface for several centimeters (a very strong positive result).

A general reaction accompanies the local reactions. This includes fever, aches, headaches, muscle pains, numbness, sore hands, swollen hands, and soreness throughout the lymphatic system and in the lymph vessels. The local and general reactions do not depend only on the dose of brucellin PS, but, to an equal extent, on the personal susceptibility of the patient and the state of the infection.

The Principles of Prophylaxis Against Brucellosis

1. Veterinary Prophylaxis

Brucellosis is an occupational disease in rural areas, industry, in the veterinary-zootechnical service itself, and, at the same time, is of epidemiological significance. Therefore, among other things, we must prohibit the use of vaccines against brucellosis in animal husbandry, since these vaccines contain living, fully virulent bacteria which have been isolated from milk, urine, and fecal matter and represent a source of infection.

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From the time when the production of S 19, which contains living, non-virulent Brucellae, was begun in Poland, we have been defending the thesis that it is not advisable to use S 19 on cattle during the 4th to the 8th month of calving. The vaccination of cows is considered impermissible from the point of view of the protection of the people. We still are not sure that S 19 is always non-virulent under all conditions. Milk cows excrete S 19 with their milk. Our research on S 19 under various conditions indicates that this vaccine is not apathogenically stabilized (Parnas, Daszkiewicz). We are developing our own nonvirulent vaccine by prolonged culturing on a bile nutrient medium. (Parnas, Daszkiewicz) This bile vaccine has almost been perfected.

The veterinary service has been designated the important social task of combating brucellosis and eliminating it on farms and in the producer cooperatives. Under our socialistic system of agriculture, the successful completion of this task depends on the method of determining the presence of brucellosis. Before the war, we, in this country, made the same error as was being made in the diagnosis of brucellosis among human patients, i.e., the agglutination reaction was not used to detect the disease in cattle. For this reason there was always a certain number of brucellosis carriers, despite the fact that cattle that reacted positively were removed.

The adoption of our diagnostic plan for brucellosis among cattle and other animals by the State Veterinary Institute and the inclusion of the complement fixation in the diagnostic procedures has made possible the detection of a greater number of carriers. These two reactions are still not used sufficiently on the PGRs and producer cooperatives. We also consider it necessary to use brucellin PS. This proposal is based on the fact that the combined use of both these reactions, together with the Burnet reaction will increase the possibility of detecting and eliminating carriers. We are of the opinion that the model dairies producing milk for children should be investigated with the aid of this type of diagnostic reaction. After the detection of brucellosis carriers, it is necessary to eliminate them from the farms and either consign them to the slaughterhouse or collect them on special farms designated for the concentration of cattle and other animals infected with brucellosis. Both of these courses are possible only under a socialistic system of animal husbandry -- they could not be realized under a private-ownership system of animal husbandry. The thorough disinfection of cattle sheds, control of rodents and flies, and exclusion of cattle from the cattle sheds without a preliminary examination for brucellosis are necessary supplemental measures in the struggle against brucellosis on the farms. The cattle collected on the "brucellosis" farms are slowly eliminated so that after a certain period, the farms will become free of brucellosis.

2. Medical Prophylaxis

Instructing the workers about the sources of brucellosis infection and the methods of defense against it is of decisive significance. In this connection, the institute has published popular-science brochures for workers on the PGRs in the meat and dairy industries and for the members of the veterinary-zootechnical service. Thanks to this, a great deal of attention is being devoted to combating brucellosis as an occupational disease.

The basis of personal protection for workers is the systematic observance of hygienic principles and of safe work practices in places where sources of brucellosis infection exist. Personal defense precautions include special clothing, rubber gloves, boots, berets for the head, observance of the principles of personal cleanliness, and no smoking during work. The greatest difficulty is presented by the protection of the hands. Thick rubber gloves do not rip, but are inconvenient and uncomfortable, while thin rubber gloves rip easily, especially where the work requires some exertion, i.e., during veterinary maternity work. The use of protective creams for the hands has been proposed. We investigated some of the creams which contained bactericidal and bacteriostatic substances, i.e.,

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silver, iodine, streptomycin, and sulfadiazine, using experimental animals under conditions simulating those found on the skin during work. The protective creams did not afford 100% protection to the skin. We found that 50% of the 25 experimental animals contracted brucellosis through the cream barrier on the skin and developed general infections (Parnas, Glinkowa). Therefore, at present, we recommend only rubber gloves as 100% effective. In the USSR, protective vaccines containing nonvirulent living strains of *Br. bovis* (Zdrodovskiy) are used on workers who are particularly exposed to brucellosis. We submitted a proposal to the Ministry of Health that workers on PGRs which are severely infected with brucellosis, and veterinary physicians, zootechnicians, feldshers, and veterinary sanitation workers who come in contact with infected animals in their daily work be given a yearly vaccination against brucellosis.

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